<Advanced Research Center for Beam Science> Structural Molecular Biology

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- Structural Molecular Biology -

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Scope of Research

The research activities in this laboratory are performed for X-ray structural analyses of biological macromolecules and the investigation of the electronic state in materials as follows: The main subjects of the biomacromolecular crystallography are crystallographic studies on the reaction mechanism of enzymes, the relationship between the multiform conformation and the functional variety of proteins, and the mechanism of thermostabilization of proteins. In the investigation of the chemical state in materials, the characteristics of the chemical bonding in the atom and molecules are investigated in detail using a newly developed X-ray spectromator with a high-resolution in order to elucidate the property of materials. The theoretical analysis of the electronic states with DV-Xα and WIEN2k, and the development of new typed X-ray spectrometer with ultra high-resolution have also been carried out.

Research Activities (Year 2004)

Presentations

X-Ray Crystal Structure Analysis of the Complex between Carboxypeptidase Y and a Protein Inhibitor I^C, Mima J (Kyoto University), Hayashida M, Fujii T *et al.,* Annual Meeting, Jpn Soc. Biosci. Biotech. Agrochem., 29 March.

Crystallization and Preliminary X-ray Analysis of Carboxypeptidase Y Inhibitor I^C Complexed with the Cognate Proteinase, Mima J (Kyoto University), Hayashida M, Fujii T *et al.,* Acta Crystallogr. **D60**, 1622 - 1624 (2004).

X-Ray Crystal Structure Analysis of a Complex between Carboxypeptidase Y and a Protein Inhibitor, Hata Y, Hayashida M, Fujii T *et al.,* 6th Conference, Asian Crystallogr. Assoc., 28 June.

Crystal Structure of a Protein Complex and Functional Analysis of Mutants of Multi-functional Protease Inhibitor I^C with Phospholipid Binding Activity, Mima J (Kyoto University), Hayashida M, Fujii T *et al.,* Annual Meeting, Mol. Biol. Soc. Jpn, 9 December.

The relation between X-ray emission in $LiNbO_3$ and its crystal structure, Ito Y, 18th International Conference on the Application of Accelerators in Research and Industry, Denton, Texas, USA, 10 - 15 October (invited).

X-ray emission from pyroelectric crystal with the thermal process, Ito Y and Yoshikado S (Doshisha University), 60th workshop of the bulk growth in the Japanese Association for Crystal Growth Cooperation, Tokyo, Japan, 13 Feburary (invited).

Grants

Hata Y, Structural Analyses of Gene-products Involved in Protein Structure Formation, Protein 3000 Project, 1 April 2002 - 31 March 2007.

Sanjoh A (Protein Wave Corporation), Hata Y *et al.,* Studies and Developments on Practical Use of Devices for Growth of Protein Crystals Suitable for Ultra-high Resolution X-ray Analysis, Grant for Spport of Studies and Developments by Cooperation of Industry, Academic and Public, Kyoto Sangyo 21 Foundation, 1 April 2004 - 31 March 2007.

Structure-based Exploration of Functional Sites in the Aspartase Family

The crystal structure of the thermostable aspartase from *Bacillus* sp. YM55-1 was solved and refined for 2.5 Å resolution data. The enzyme is a homotetramer with subunits composed of three domains. It exhibits no allosteric effects, in contrast to the *E. coli* aspartase which is activated by divalent metal cation and L-aspartate. The overall folding of the present enzyme subunit is similar to those of the *E. coli* aspartase and the *E. coli* fumarase C, both of which belong to the same superfamily with the present enzyme.

In order to observe substantial structural differences in local sites, plots of the r.m.s. C^{α}-deviation over five consecutive residues after C^{α}-fitting in this region between two structures are very effective. The local structural comparison of these three enzymes revealed seven structurally different regions (Figure 1).

Five of the regions were located around putative functional sites, suggesting the involvement of these regions into the functions characteristic of the enzymes.

Of these regions, the region of residues 96–100 is proposed as a part of the recognition site of the α-amino group in L-aspartate for aspartase and the hydroxyl group in L-malate for fumarase. The region of residues 315–323 is a flexible but well sequence-conserved loop that is suggested to be involved in the catalytic reaction. The region of residues 123–128 corresponds to a part of the putative activator-binding site in the *E. coli* fumarase C. The region in the *Bacillus* aspartase, however, adopts a main-chain conformation which prevents the activator binding. The regions of residues 228–241 and 265–272, which form a part of the active-site wall, are suggested to be involved in the allosteric activation of the *E. coli* aspartase by the binding of the metal ion and the activator.

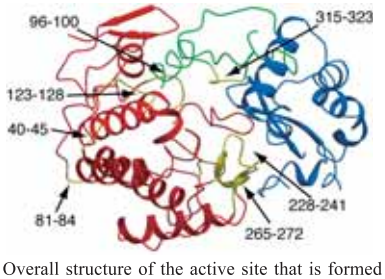

Figure 1. Overall structure of the active site that is formed by three subunits, A-, B-, and C-chains, which are colored green, blue, and red, respectively. Structurally different regions are colored yellow.

Docking Model of the Substrate into the Active Site of the Asparatase

We have made a structural model of the complex in which a substrate L-aspartate was manually docked into the active site of *Bacillus* sp. YM55-1 aspartase as followings (Figure 2).

The most conformationally different region in the vicinity of the active site is the region of residues 96–100, which is the sole region in which the structure of both the *Bacillus* aspartase and the *E. coli* aspartase differs from that of the *E. coli* fumarase. The amino acid sequence of the region is completely conserved among all aspartases, while partly different sequence is conserved for the corresponding region among all fumarases. Considering these findings and the characteristics of the functional groups bonding to the α-atom of substrates, we could expect this region to be the site recognizing the $-NH_3^+$ group of L-aspartate in aspartase or the – OH group of L-malate in fumarase.

Because the substrates of aspartase and fumarase have two carboxyl groups as a common structural feature, positively charged residues have been suggested to stabilize these negatively charged groups. In the *Bacillus* aspartase, Lys324 is only one positively charged residue in the active-site cleft and the putative binding residue of one carboxylate of L-aspartate. The site of Ser140 and Thr141, which corresponds to one of binding sites for carboxyl group of the citrate in the citrate-fumarase complex, is just at the N-terminal end of α-helix 6. It is possible that the other carboxylate of the substrate bind to this site through the positively charged environment caused by the dipole moment of α-helix.

Finally, the substrate model was docked into the active site on the computer graphics so as to satisfy the proposals that the α-amino group may be recognized by Asp142, His188, and Gly98 or Thr101 and that the α- and β-carboxyl groups bind to Lys324 and Ser140 with the dipole moment of α-helix, respectively.

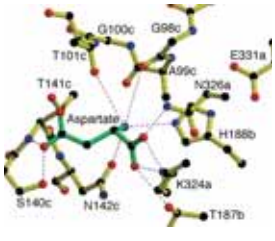

Figure 2. The putative binding mode of substrate L-aspartate in the active site. The docked model of the substrate is represented by green bonds. Putative hydrogen bonds are shown by broken lines.

Ito Y, Development of Basic Technologies for New Functional Particle Materials, Kyoto Prefecture Collaboration of Regional Entities for the Advancement of Technological Excellence, JST, 2004 - 2008.

Fujii T, Elucidation of mechanism of structural change in functional sites of aspartase, Grant-in-Aid for Young Scientists (B), 1 April 2003 - 31 March 2005.